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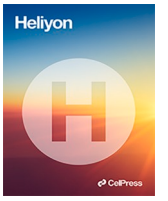

Research article

Quantitative microbial risk assessment of gastrointestinal illness due to recreational exposure to *E. coli* and *enterococci* on the southern coasts of the Caspian Sea

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A R T I C L E I N F O

Keywords:
Monte Carlo method
Bathing beaches
Enterococcus
Escherichia coli

A B S T R A C T

Background: Gastrointestinal illness refers to a broad range of diseases that affect the digestive system, including infections caused by bacteria, viruses, and parasites. Quantitative Microbial Risk Assessment (QMRA) is a powerful tool used to evaluate the risks associated with microbial pathogens in various environments. The main objective of this study was to conduct a quantitative assessment of gastrointestinal illnesses that occur as a result of exposure to *E. coli* and enterococci during recreational activities on the southern coasts of the Caspian Sea.
Methods: Samples were collected from the recreational beaches along the border line of the Caspian Sea. The samples were analyzed for the presence and enumeration of *E. coli* and enterococci using the microplate method and membrane filtration techniques. Then, the annual and daily infection risks were computed using the Monte Carlo simulation approach.
Results: The results revealed that the risk of daily and annual infections on the coasts of Babolsar was higher than that on the coasts of Sari. Also, in the recreational waters of these beaches, the risk of infection by enterococci was higher than that posed by *E. coli*. In Babolsar, the average annual infection risk caused by *E. coli* and enterococci was 0.365 and 1 for children and 0.181 and 0.986 for adults. Also, in Sari, the average annual infection risk caused by *E. coli* and enterococci was 0.060 and 0.908 for children and 0.027 and 0.815 for adults. In addition, children were more likely than adults to become infected.
Conclusion: In light of the study's findings, due to the entry of untreated urban wastewater into the southern part of the Caspian Sea (northern Iran) and the high risk of infectious diseases for children, more control and health measures are necessary for children's swimming.

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1. Introduction

One of the main issues in public health is fecal pollution of water sources, especially recreational water. The recreational and health status of a beach depends on the amount of pollutants and infection sources [1,2]. Eye, ear, skin, and gastrointestinal infections have been reported among people who swim on recreational beaches, and in these recreational waters, the sources of pollution, which could be point and/or non-point, have not been well identified yet [3–5]. Indeed, several studies have shown that outbreaks of waterborne diseases, in particular intestinal viruses and protozoa, are caused by direct contact or unintentional consumption of contaminated water [6–9]. Untreated wastewater is frequently dumped into seas or surface water bodies from a variety of sources, including homes, hospitals, businesses, and farms. Practice poses a potential fecal-oral transmission route for individuals swimming at beaches. Monitoring usually includes measuring two indicator bacteria, intestinal enterococci and *Escherichia coli* (*E. coli*), to determine whether fecal contamination is present. These microorganisms are trustworthy markers of the degree of fecal pollution in the water [10–12]. The Caspian Sea's coastline waters have been contaminated due to the unsanitary discharge of industrial, household, and agricultural waste into the body of water. This pollution poses a threat to the health of tourists and swimmers who come into contact with these contaminated waters. Several studies have indicated that microbial and chemical contamination is present in all coastal areas of the Mazandaran Province, located along the northern border of Iran [13–15]. In Europe, sanitary inspections regarding fecal contamination of recreational beaches are used to determine if they meet recreational water quality guidelines [16]. Fecal indicator bacteria are commonly used to determine microbial water quality standards because method of their detection is simple, inexpensive, and of little complexity. The presence of microorganisms such as *E. coli*, intestinal enterococci, total and fecal coliforms indicates fecal contamination of warm-blooded animals. Among these microorganisms, *E. coli* and intestinal enterococci are special indicators of fecal contamination by human wastes [17–19]. The United States Environmental Protection Agency (U.S. EPA) adopts various thresholds for enterococci and *E. coli* depending on the kind of recreational water. Enterococci are utilized for freshwater and ocean, while E. Coli is used to signal the health risk in recreational freshwater. According to the criteria announced by the U.S. EPA, the geometric densities of enterococci and *E. coli* for recreational marine and freshwaters are 35 and 126 CFU 100 mL^{-1}, respectively. The National Environmental and Epidemiological Evaluation of Recreational (NEEAR) Water Studies has promulgated the threshold rate range of NEEAR-gastrointestinal disease as 32 or 36 people per 1000 bathers [19–21]. Given the high expense of epidemiological investigations, QMRA is generally worked out to assess public exposure to pathogenic infections and establish a dose-response relationship. The QMRA method was used to determine the health risks of exposure to *E. coli* and enterococci during recreational activities at those coasts that are affected by sewage effluents [21,22]. In many studies, *E. coli* and enterococci have been used as indicators of marine recreational water safety risk. Bonamano et al. reported the contamination of a local recreational area of an Italian coast and presented the distribution data of *E. coli* in their study. In their report, it was stated that during the summer, there was a significant presence of *E. coli* in the recreational beaches. This occurrence was linked to the overflow originating from wastewater treatment facilities, as per their findings [23,24]. Additionally, considering the restrictions on *E. coli* as an ideal indicator for recreational coast water quality, the U.S. EPA recommended the use of enterococci as a safety indicator for marine recreational beaches [21]. Environmental health units conduct sampling and monitoring activities to assess the quality of coastal water during the spring and summer seasons, which coincide with the peak tourist influx to the area. However, it is unfortunate that there is a lack of comprehensive studies focusing on risk assessment in this regard. This gap in knowledge hinders our understanding of the potential risks and health implications associated with swimming in these waters for the local population.

The main aims of the research are as follows: 1- To assess the health risk of recreational waters in terms of *E. coli* and enterococcus bacteria in certain coastal cities located in northern Iran along the Caspian Sea border (specifically Babolsar, southwest of the Caspian Sea, and Sari, southeast of the Caspian Sea). The southern shores of the Caspian Sea, particularly the swimming beaches of Mazandaran province, are susceptible to various types of pollution, with microbial pollution posing a significant risk of illness to swimmers. 2- To generate results that can be applied to enhance the development of criteria for recreational water quality and assess the conditions of recreational beaches.

2. Materials and methods

2.1. Study area

This study took place within the coastal cities of Babolsar and Sari, located in the northern region of Iran near the southern shores of the Caspian Sea. These cities have, respectively, 25 and 20 km of pleasant sandy beaches and are the most famous beach resorts in the country. As a result, the entrance of storms and the intentional/unintentional discharge of untreated sewage, as well as the leakage of leachate from landfilled or dumped solid waste, result in the pollution of sea water. This in turn not only poses adverse consequences for environmental quality and public health but also for the economic status of these recreational areas.

2.2. Analysis of fecal indicators

2.2.1. Sample collection

Sampling was carried out monthly throughout the year and at all sites (8 beach sites in Babolsar and 7 beach sites in Sari). Totally, 96 samples were taken from the coastal beach of Babolsar. Similarly, 84 samples were obtained from Sari. Samples were taken at a depth ranging from 20 to 50 cm beneath the water's surface. Samples were collected from each beach on a monthly basis over the

course of the entire year.

2.2.2. Sample preparation

Sterile bottles were used to collect samples. The samples were immediately sent to the laboratory in a cooled, sterile container and analyzed within 24 h. Some chemical and physical characteristics of the water, such as pH and temperature, were measured and assessed. The average ambient temperature and average water temperature during spring and summer were recorded as ranging from 25 to 38 °C and 16–21 °C, respectively.

2.2.3. Sample analysis

The standard procedures ISO 7899-1 (1999) and ISO 9308-3 (1998) were used to analyze the intestinal enterococci and *E. coli*, respectively. The microplate method was used to count cell density, which was counted as the most probable number (MPN)/100 mL of sample. The formation of gas and/or the development of turbidity were considered to indicate the presence of *E. coli*. Likewise, the membrane filter (MF) technique was used to count enterococci. After a period of 24–48 h, distinct colonies exhibiting a characteristic brown to black appearance were counted [25,26].

2.3. Quantitative microbial risk assessment (QMRA)

The risk of infection and its role in pathogenicity through swimming activities in these recreational areas were assessed by focusing on the microbiological quality of the water, and the data were analyzed through quantitative microbial risk assessment (Eqs. (1)–(3)).

$$D_{Pat} = C_{Path} \times V_{ing} \tag{1}$$

$$\mathrm{p\_(inf)} = 1\text{-}\left[1 + \frac{D_{pat}}{N_{50}}\left(2^{\frac{1}{\alpha}} - 1\right)\right]^{-\alpha} \tag{2}$$

$$\mathrm{p_{(n)}} = 1\text{-}\left(1\text{-}\,\mathrm{p_{inf}}\right)^{n} \tag{3}$$

in Eq. [1], D_{path} is the received dose of *E. coli* or enterococci, *Ving* is the randomly swallowed volume of water during swimming, and C_{path} is the concentration of the studied microbial pathogen in water. It has been approximated that children consume approximately 37 mL of water per swimming event, while adults consume around 16 mL [27].

The study took into account individuals under 15 years of age as children and those between 15 and 80 years old as adults. In Equation [2], p_{inf} represents the probability of daily infection risk, α is the parametric slope, and N_{50} is the average dose to cause an infection (for *E.coli*; N_{50} = 2.55E+06, α = 0.175, and for enterococci; N_{50} = 5.99E+04, α = 0.16). $P_{(n)}$ is the probability of annual infection, and *n* is the exposure time (day) per year [28–30].

2.4. Statistical analysis

Monte Carlo simulations (MCS) were performed through the probability distribution function. In the field of QMRA, it is customary to utilize MCS as a means to effectively depict the variability or uncertainty of parameters. Following the development of the model, MCS was performed with Crystal Ball software added to Microsoft Excel software to quantify parameters and analyze the data [31,32]. The amount of swallowed water and the concentration of *E.coli* and enterococci in the water samples obtained from the studied recreational beaches were included in the model.

3. Results

Using the Beta Poisson model in QMRA, it is possible to compute the probability of infection by utilizing the average levels of *E. coli* and enterococci concentrations mentioned in Eq [2].

Table 1
The risk of daily and annual infection *E. coli* for children and adults on Babolsar recreational beaches.

Parameter	Child		Adult	
	[a]DI	[b]AI	DI	AI
Mean	4.41×10^{-3}	3.65×10^{-1}	1.93×10^{-3}	1.81×10^{-1}
[c]SD	1.06×10^{-3}	7.05×10^{-2}	4.61×10^{-4}	3.94×10^{-2}
10 %	3.06×10^{-3}	2.73×10^{-1}	1.33×10^{-3}	1.29×10^{-1}
30 %	3.87×10^{-3}	3.32×10^{-1}	1.69×10^{-3}	1.61×10^{-1}
60 %	4.68×10^{-3}	3.86×10^{-1}	2.05×10^{-3}	1.92×10^{-1}
90 %	5.77×10^{-3}	4.52×10^{-1}	2.52×10^{-3}	2.31×10^{-1}

[a] DI: Daily infection.
[b] AI: Annual infection.
[c] SD: Standard Deviation.

The probability of infection was in turn considered to calculate the probability of annual infection using Eq. [3], as summarized in Tables 1–4, which demonstrate the possibility of daily and annual infection risk caused by swimming in coastal waters on the studied coastlines in children and adults.

Due to the fact that the concentrations of *Enterococci* and *E.coli* are distributed non-parametrically on the recreational beaches of these two cities, the 10th, 30th, 60th, and 90th percentiles and the mean and standard deviation of daily and annual infection risk were determined for the concentrations of enterococci and *E. coli* observed in children and adults on recreational beaches (Tables 1–4).

As shown in Tables 1 and 3, the average daily infection risk of *E. coli* for adults on the Babolsar and Sari coastlines was 1.93×10^{-3} and 2.66×10^{-4}, respectively, and the average annual infection risk was 1.81×10^{-1} and 2.72×10^{-2}, respectively. The average risk of *E. coli* for children in Babolsar and Sari coastlines as a daily infection risk was 4.41×10^{-3} and 6.08×10^{-4}, respectively, and the annual infection risk was 3.65×10^{-1} and 6.07×10^{-2}, respectively. Table 2 shows the average risk of intestinal enterococci for adults and children on Babolsar coastlines. In this case, the daily infection risk was 4.30×10^{-2} and 8.38×10^{-2}, respectively, and the annual infection risk was 9.86×10^{-1} and 1, respectively. The mean risk of intestinal enterococci for adults and children in Sari beaches in terms of daily infection risk was 3.96×10^{-2} and 7.24×10^{-2}, respectively, and the annual infection risk was 8.15×10^{-1} and 9.08×10^{-1}, respectively (Table 4).

Figs. 1–4 depict the risk of annual pathogenicity of the 90th percentile of *E.coli* is 2.31×10^{-1} for adults and 4.52×10^{-1} for children who have fun on the coastline of Babolsar. Also, 4.73×10^{-2} for adults and 1.05×10^{-1} for children who are having fun on the coastline of Sari. Regarding intestinal *enterococci*, the risk of annual pathogenicity in the 90th percentile is 9.96×10^{-1} for adults and 1children who have fun on the recreational beaches of Babolsar and 9.99×10^{-1} for adults and 1children who have fun on the beaches of Sari. It should be noted that the upper border shows the blue diagrams to the left of the 5th percentile, and the lower border shows the blue diagrams to the right of the 95th percentile.

4. Discussion

The Caspian Sea, renowned as the largest landlocked body of water globally, features southern shores that hold significant value as recreational zones for both residents and visitors. Nonetheless, the existence of harmful microorganisms in the seawater presents a notable hazard to the well-being of the general public. Understanding the risks associated with gastrointestinal illness in this region is crucial for implementing effective management strategies to protect the wellbeing of the population. The risks associated with gastrointestinal illness in sea water are multi-faceted. Factors such as pollution, improper waste disposal, and the presence of untreated sewage can contribute to the contamination of the water with pathogens. Additionally, natural phenomena such as algal blooms can further exacerbate the risk by altering the water quality and promoting the growth of harmful bacteria. In many parts of the world, *E. coli* and enterococci are used as indicators of water quality to indicate fecal pollution and recreational beach water quality management [33].

Due to the fact that *E. coli* and intestinal enterococci data were nonparametrically distributed on recreational beaches, MCS was used to investigate their changes in this study. MCS are reliable for determining E.cola and enterococci concentration variables in QMRA. The QMRA method is used to determine the health risk of water consumption due to the risk of water resources, recreational waters, and wastewater reuse [34–36]. The risk infection of enterococci is about 2–11 times higher than *E. coli* on these recreational beaches. Based on the data in Tables 1 and 3, the average annual infection risk of *E. coli* for children at the recreational beaches of Babolsar and Sari was 3.65×10^{-1} and 6.07×10^{-2}, respectively. Meanwhile, for adults, it was 1.81×10^{-1} and 2.72×10^{-2}, respectively. These findings suggest that the risk of infection is higher for children compared to adults. Rafiei et al. [37] reported in their study that the risk of annual infection for adults was lower than that for children. Also, the risk infection of *E. coli* on Babolsar recreational beaches is higher than Sari beaches. Because children have weaker immune systems and consume higher amounts of water in recreational waters, as a result of been the most sensitive and vulnerable people who are exposed to microbial contamination. For this reason, quantitative microbial risk assessment in recreational beach water is important to determine the unsanitary discharge of wastewater into these waters [38,39]. The risk associated with *E. coli* was evaluated by comparing it to the standards set by the World Health Organization (WHO) and the EPA. The risk of diarrheagenic *E. coli* in sewage-contaminated water was assessed by the WHO and EPA through evaluating the pathogen levels in human sewage or studying the occurrence of waterborne diseases. Their assessment indicated that the risk associated with *E. coli* in water contaminated with sewage ranges from 10^{-5} to 10^{-6}, representing about 0.04 % of the total risk of waterborne illnesses. Based on the mean daily infection risk, the order of enterococci daily infection risk for children and adults is as follows: Babolsar recreational beaches (8.38×10^{-2} and 4.30×10^{-2}) > Sari (7.24×10^{-2} and 3.96×10^{-2})

Table 2
The risk of daily and annual infection enterococci for children and adults on Babolsar recreational beaches.

Parameter	Child		Adult	
	DI	AI	DI	AI
Mean	8.38×10^{-2}	1	4.30×10^{-2}	9.86×10^{-1}
SD	1.21×10^{-2}	1.39×10^{-3}	7.20×10^{-3}	1.40×10^{-2}
10 %	6.82×10^{-2}	9.99×10^{-1}	3.36×10^{-2}	9.71×10^{-1}
30 %	7.78×10^{-2}	1	3.93×10^{-2}	9.85×10^{-1}
60 %	8.73×10^{-2}	1	4.49×10^{-2}	9.92×10^{-1}
90 %	9.91×10^{-2}	1	5.21×10^{-2}	9.96×10^{-1}

Table 3
The risk of daily and annual infection *E. coli* for children and adults on Sari recreational beaches.

Parameter	Child		Adult	
	DI	AI	DI	AI
Mean	6.08×10^{-4}	6.07×10^{-2}	2.66×10^{-4}	2.72×10^{-2}
SD	3.55×10^{-4}	3.47×10^{-2}	1.55×10^{-4}	1.56×10^{-2}
10 %	1.52×10^{-4}	1.57×10^{-2}	6.84×10^{-5}	7.09×10^{-3}
30 %	4.21×10^{-4}	4.29×10^{-2}	1.85×10^{-4}	1.90×10^{-2}
60 %	6.97×10^{-4}	7.00×10^{-2}	3.06×10^{-4}	3.13×10^{-2}
90 %	1.06×10^{-3}	1.05×10^{-1}	4.66×10^{-4}	4.73×10^{-2}

Table 4
The risk of daily and annual infection enterococci for children and adults on Sari recreational beaches.

Parameter	Children		Adults	
	DI	AI	DI	AI
Mean	7.24×10^{-2}	9.08×10^{-1}	3.96×10^{-2}	8.15×10^{-1}
SD	3.26×10^{-2}	3.04×10^{-2}	1.82×10^{-2}	1.01×10^{-1}
10 %	3.04×10^{-2}	9.59×10^{-1}	1.25×10^{-2}	7.31×10^{-1}
30 %	5.91×10^{-2}	9.98×10^{-1}	2.85×10^{-2}	9.50×10^{-1}
60 %	8.37×10^{-2}	1	4.25×10^{-2}	9.89×10^{-1}
90 %	1.10×10^{-1}	1	5.90×10^{-2}	9.99×10^{-1}

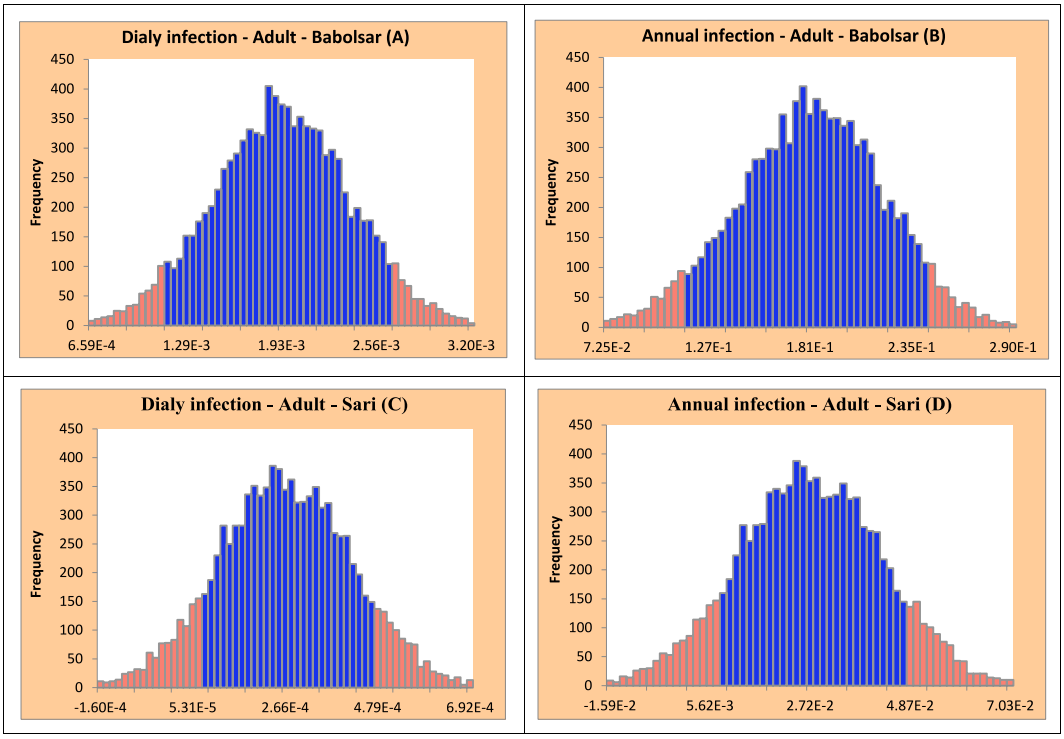

Fig. 1. The risk of daily and annual infection *E.coli* for adults ingested coastal water on Babolsar (A and B) and Sari (C and D) recreational beaches.

recreational beaches. The annual infection risk of enterococci in Babolsar recreational beaches is the same as in Sari recreational beaches (Tables 2 and 4). Furthermore, when categorized by age group, the annual risk of infection from enterococci was found to exceed that of *E. coli*. The decomposition rate of *E. coli* is higher in seawater compared to freshwater due to its lower resistance to osmotic shock. *E. coli* requires a higher level of intracellular osmotic pressure to sustain its survival in seawater. As a result, the conditions in seawater lead to a faster breakdown of *E. coli* compared to freshwater. Consequently, the US EPA recommends using *E. coli* less frequently for monitoring beach recreational water quality. On the other hand, enterococci demonstrate greater resistance to osmotic shock than *E. coli*. As a result, a higher concentration of enterococci is utilized to assess the quality of seawater [8]. The greater

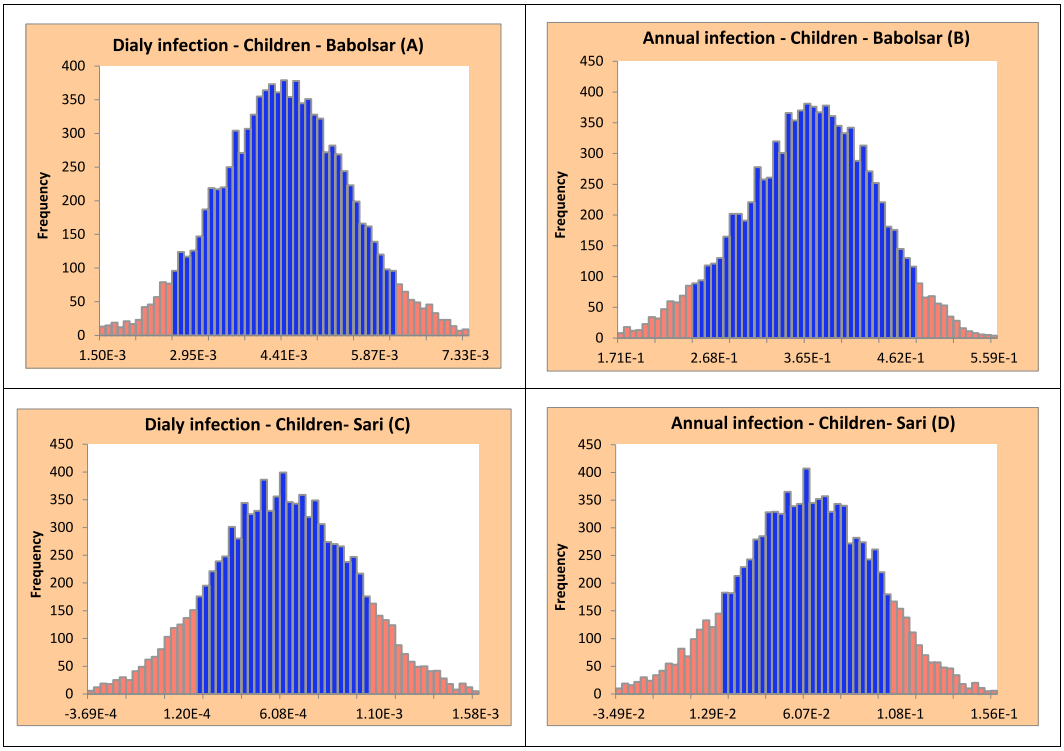

Fig. 2. The risk of daily and annual infection *E.coli* for Children ingested coastal water on Babolsar (A and B) and Sari (C and D) recreational beaches.

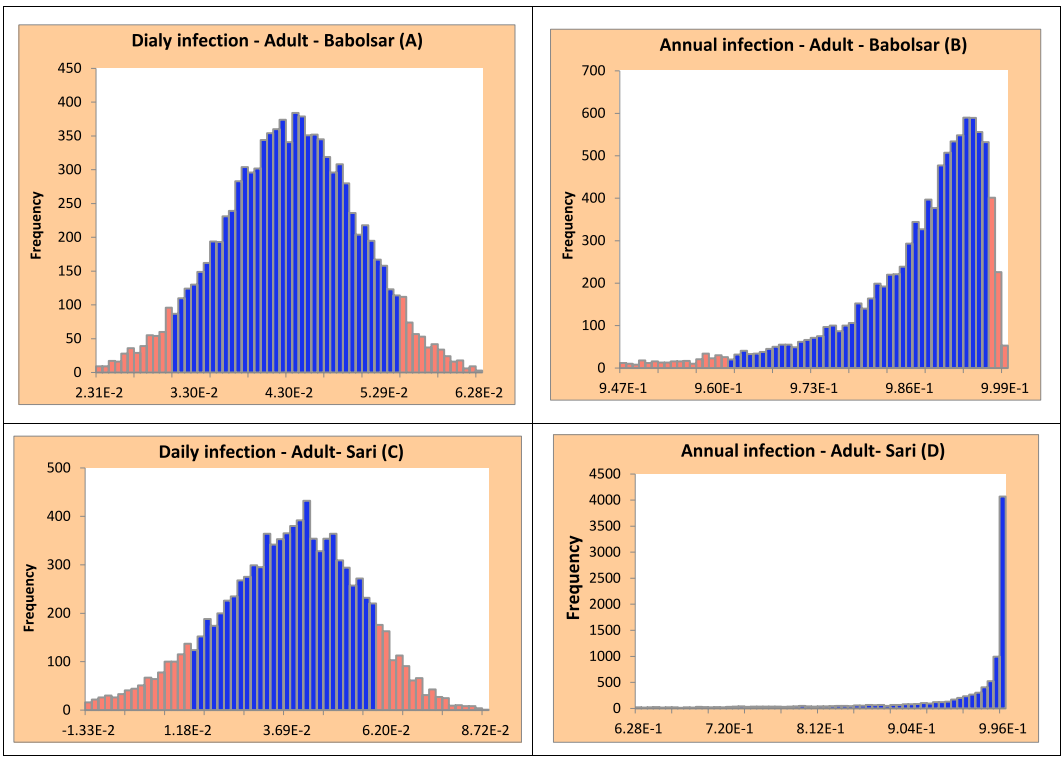

Fig. 3. The risk of daily and annual infection enterococci for adults ingested coastal water on Babolsar (A and B) and Sari (C and D) recreational beaches.

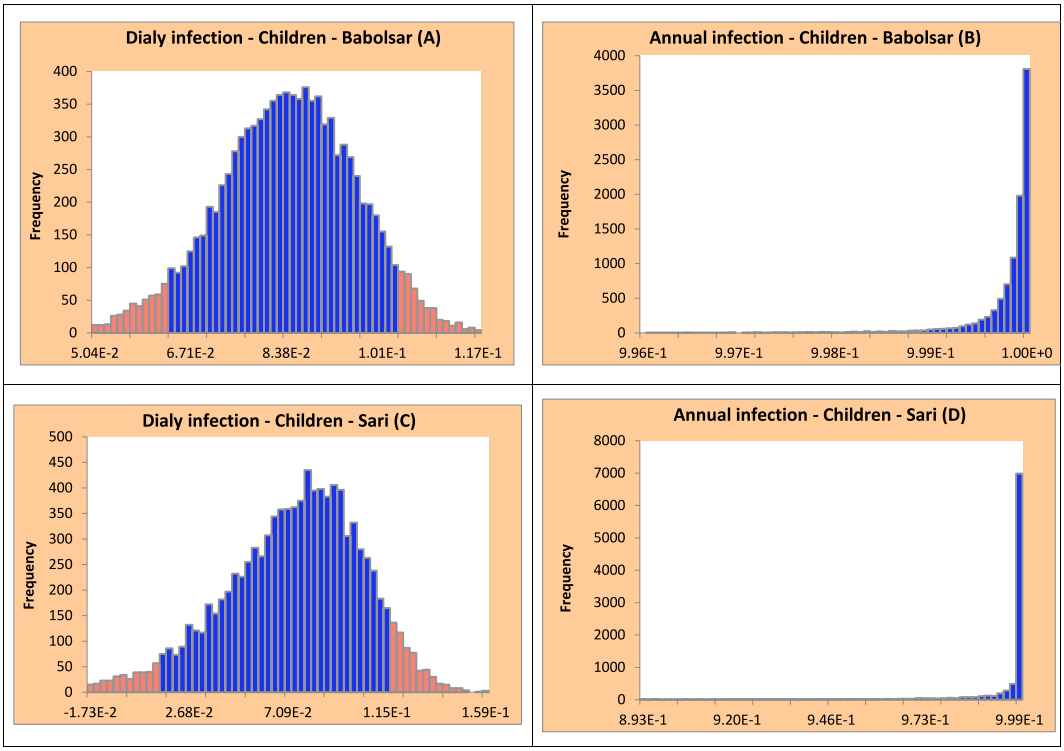

Fig. 4. The risk of daily and annual infection enterococci for Children ingested coastal water on Babolsar (A and B) and Sari (C and D) recreational beaches.

risk of pollution on the recreational beaches of Babolsar is due to the discharge of agricultural and municipal wastewater into the sea. In studies conducted by Hamilton et al. and Boehm et al. conducted in recreational waters in Avalon Bay, California, the concentration of E.cola was high, and they stated that the main reason was the entry of unsanitary sewage into these recreational beaches water [40, 41]. Many studies have found that heavy rainfall and the discharge of municipal and agricultural wastewater into the coastal oceans transport large amounts of pathogenic microorganisms to recreational waters in those areas [42,43]. One of the effective solutions and control strategies to reduce the entry of runoff and sewage to recreational beaches is to use low-flow diversions and wetland establishments and also improve wastewater treatment systems [44]. The main cause of fecal contamination in Iran recreational beaches is the uncontrolled release of human sewage and agricultural runoff. It is advised that following heavy rainfall causing an increase in fecal contamination in recreational waters, for the sake of public health, swimming should be temporarily halted at beaches with moderate risk levels. However, contact with recreational waters reveals a variety of reports of skin, eye, and hearing problems. We can also use this data to assess the risks of water-borne pathogens of non-fecal origin. In the current situation, such risk assessment is limited due to the shortage of dose-response information [45].

In the current study, the main limitations included 1- For future research, it is advisable to utilize indicator organisms in risk assessment that accurately represent the actual risks associated with various enteric pathogens, including viruses or protozoa. 2- Samples were also collected in months when swimming is less common, potentially indicating a lower correlation with recreational water exposure compared to the swimming season. 3- One major drawback of this study is the inability to categorize risk levels for different genders and age groups prone to different diseases, which prevented the opportunity to perform a sensitivity analysis.

5. Conclusions

This research was conducted to evaluate the health risks of recreational swimming pools on the recreational beaches of two cities on the southern coasts of the Caspian Sea using QMRA. QMRA is an effective tool that has a high ability to estimate and predict the probability of infection and disease in human communities exposed to water with pathogenic microorganisms. The QMRA results provide information on the quality of recreational waters and health hazards in the study population. Also, the results retrieved from the potential risk assessment for the recreational beaches can be used as a guideline for recreational water management in Iran. The risk of daily and annual infections for recreational swimmers on the coast of Babolsar (southwest of the Caspian Sea) is higher than that for the coast of Sari (southeast of the Caspian Sea). Accordingly, environmental management and public health improvement solutions should be implemented at medium-risk recreational beaches.

Ethical issues

This research is associated with project number IR.SBMU.RETECH.REC.1400/4618 from the Student Research Committee at Shahid Beheshti University of Medical Sciences in Tehran, Iran. The authors verify that all data obtained during the research matches the information presented in the manuscript, and no data from the study has been or will be released elsewhere.

Data availability

All data are included in article/supp. material/referenced in the article.

CRediT authorship contribution statement

Hadi Niknejad: Data curation. **Somayeh Hoseinvandtabar:** Methodology, Investigation. **Maryam Panahandeh:** Writing – review & editing. **Fathollah Gholami-Borujeni:** Investigation, Funding acquisition. **Reihaneh Janipoor:** Software, Project administration. **Roghayeh Abedi Sarvestani:** Writing – original draft, Visualization. **Reza Saeedi:** Investigation, Funding acquisition, Formal analysis. **Mohsen Hesami Arani:** Funding acquisition, Conceptualization. **Mehrnoosh Abtahi:** Writing – review & editing, Visualization, Supervision. **Mohammad Rafiee:** Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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